REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1-2, 5-14, 16 and 23-24 are pending. A nonfinal Office Action was mailed on September 20, 2007. Its mailing was premature because Applicants requested a three-month suspension of action to provide additional evidence in support of patentability. Consideration of the following remarks and attachments is requested.

The latest citation of the Examiner (i.e., the Mikawa reference) indicates that a further explanation of Applicants' invention and contrast with the prior art might be helpful to avoid ignoring or misunderstanding critical aspects of the invention. These are precisely the aspects which have led to the high interest in Applicants' method. It has been used for quantifying the effect of psychological stress in a very wide variety of situations from housing and capture of wild animals, to coaching of athletes. Indeed, the Mikawa reference is not even to do with the same technical problem. Mikawa studied the effect of surgery under general anaesthesia on neutrophils of neonates and infants; not attempting to quantify psychological stress. It is simply not correct to equate the terms "psychological stressor" and "surgery" as the Examiner has done. Anticipation of surgery could well be a psychological stressor for some pre-operative patients, but Mikawa were not focusing on this but perioperative changes, more particularly variation in neutrophil count and activity over the time period immediately surrounding and including actual surgery.

It would appear that the Examiner has recognized the technical deficiencies of the Tsukamoto reference as prior art, but the Mikawa reference cannot be viewed as a better citation. Rather it is simply not relevant prior art. As will be expanded upon below, the Examiner is making a fundamental mistake in thinking that the Mikawa reference somehow provides teaching relevant to quantifying the effect of any form of psychological stress; it merely extends to infants and neonates a prior recognized immunological effect of undergoing surgery ("It is well established that both anaesthesia and surgery cause transient immunosuppresssion . . .", bottom paragraph of the right column on the front page of the Mikawa reference).

The method of the invention is the basis of a spin-out company and has now been described in a number of peer-reviewed papers, starting with McLaren et al., "Leukocyte coping capacity: A novel technique for measuring the stress response in vertebrates," Exp. Physiol. 88:541-546 (2003). The latest such publication is Moorhouse et al. "Physiological consequences of a captive conditions in water voles (*Arvicola terrrestris*)," J. Zoology 271:19-26 (2007), a copy of which is attached. This paper again focuses on use of the method in relation to handling of wild animals, but the specification illustrates use of the same method to quantify the ability of humans to cope with a diverse range of psychological stressors including horror film viewing and mental test under time pressure. Both the commercial and academic interest in the claimed method do not sit easily with the Examiner's suggestion that it is obvious.

Applicants submit that proper consideration of the invention in terms of its essential features can only rightly lead to the conclusion that it embodies a new and inventive concept. A feature of the method is use of inducer-stimulated superoxide production by neutrophils (e.g., PMA-challenge of neutrophils), but this feature is not the whole invention. Rather the invention resides in the context of the use of such neutrophil stimulation to give a highly physiologically relevant and convenient test for the effect of psychological stress. This is discussed further in the accompanying declaration of the inventor Dr. Rubina Mian. With a view to aiding the Examiner understand better the true distinctions from prior art methods, there is also set forth below a review of essential features of the invention with reference to the language of claim 1.

Essential Elements/Steps of the Invention

1. Applicants would like to emphasize that their invention is to provide a measure of the effect of psychological stress. The preamble of claim 1 requires "determining whether an individual, which is a mammal or bird, is experiencing changed physiological status arising from exposure to a psychological stressor." Psychological stress may have many origins, but as already noted above, it does not encompass what might be termed surgical stress or perioperative stress, which is the only subject of the Mikawa reference.

2. The method requires use of whole blood samples, which may be employed without reference to neutrophil number. It does not rely on use of a fixed number of isolated neutrophils. As expanded upon in the Declaration of Dr. Mian, this aspect of the claimed method is central to its worth but is not a feature derivable from any cited document. Mikawa used in their studies a fixed number of isolated neutrophils. The same was true of the previously cited studies of Kang. This would fit with concern over change of neutrophil/leukocyte number in test individuals under the test conditions and enables colorimetric assay of superoxide production. However, the exemplification of the subject invention shows that neutrophils can be successfully used as biomarkers of psychological stress in whole blood samples whereby they are retained in their in vivo environment. This can be enabled by moving to use of chemiluminescence assay of superoxide production coupled with PMA stimulation. These are practical considerations for operating the invention but not the invention in toto. Importantly, realization of the invention demanded a different mind set starting from considering that psychological stress produces multifaceted effects and giving precedence to this rather than fixing neutrophil number. The end result, a more physiological relevant test and hence a test giving a truly meaningful measure of coping capacity for psychological stress, was hoped for by the inventors, but was only seen to be feasible with availability of the results of first application as set out in Example 1 and published in the McLaren reference cited above.

3. In carrying out a test of the invention, a test whole blood sample is contacted with inducer (e.g., PMA) to stimulate superoxide production. This will be largely, although not exclusively, from neutrophils. There must be a basal measurement (prior to stimulation) and one or more after contact with inducer. There must necessarily be comparison with a control sample under identical conditions. This accords with claim 1(b): "determining superoxide production above basal in said test sample." However, a control need not be carried out with each test. All that is required is that a suitable control result is available for meaningful comparison, e.g., derived from one or more individuals exposed to the same regime minus a factor to be tested as a psychological stressor.

The quantification of stress requires the following calculations. With a test whole blood sample from an individual exposed to a known or putative stressor:

Ytest = Xtest-Btest

where Btest = basal production of superoxide in the test whole blood sample (without stimulant)

Xtest = production of superoxide in test whole blood sample at a chosen time point with stimulant (e.g., PMA)

and comparison with Ycontrol calculated as

Xcontrol - Bcontrol

where Bcontrol = basal production of superoxide in control whole blood sample (without stimulant)

Xcontrol = production of superoxide in the control sample at the same chosen time point with stimulation.

If Ytest = Ycontrol then the conclusion must be that the supposed stress factor has not affected the ability of blood cells (mainly neutrophils) of the test individual to produce superoxide when stimulated; the factor is not a stress factor or the individual is not significantly affected by the stress factor.

If Ytest < Ycontrol, then this is a marker of exposure of the test individual to a psychological stressor which has caused neutrophil activation. "The degree of further in vitro induced superoxide production in the test sample above basal" then observed with stimulation. For example, PMA-challenge (what might be termed residual capacity to produce in vitro stimulated superoxide) is a measure of coping capacity of the test individual for the psychological stressor of concern, i.e., there is an objective measure of the stress effect in terms of induced superoxide production (largely but not exclusively from neutrophils). This accords precisely with claim 1(c).

The important point to note is that little or no induced superoxide production in the test sample with PMA-stimulation is indicative that the neutrophils have been exhausted, or almost exhausted, of capability for superoxide production by the effects in whole blood arising from exposure to psychological stress.

Section 112 Claim Rejection

The Examiner is maintaining that claim 1(c) is unclear, but it is difficult to understand her reasoning. She appears to be referring to a second basal measurement on the test sample for the purpose of control but this is nonsensical. As indicated above, a result Xcontrol- Bcontrol is required to know the stimulated superoxide production to be expected in the absence of a suspected or known psychological stressor. This result is attained for a separate control sample. It might be attained in parallel with stimulation of superoxide production in a test sample but not necessarily. Time courses might be run as illustrated by the examples. However, it might be provided merely as written information from previous control studies. Claim 1(c) sets out the essentials for converting the residual capacity for stimulated superoxide production above basal found for a test sample to a measure of coping capacity by comparison with the equivalent result for a control sample as detailed above. It reflects standard assay practice and is considered to be clear such that it would be well understood by a person seeking to use the invention with the normal skills expected for superoxide measurement in a blood sample.

Turning now to the prior art rejections, the Examiner has now revisited the prior art twice since the first Office Action to fish out new documents to rely on and on each occasion has found an even more remote starting point! It is submitted that this in itself is suggestion that the Examiner is looking at a true invention. To quote from the Declaration of Dr. Mian, "I can see the pattern, key words are correct (e.g., neutrophils, stimulation, superoxide production) but the Examiner is not reading these in the right context. I have welcomed being more educated about surgical operations in neonates, but the studies of Mikawa do not form part of the technical background to the invention." The deficiencies of Mikawa as a prior art document to base an obviousness rejection on are further expanded upon below.

Section 103 Rejection over Mikawa et al. in view of Pfefferkorn

The Examiner starts off at the top of page 4 of the latest Office Action (now withdrawn) by stating that "a method is claimed for determining whether an individual is experiencing changed physiological status arising from exposure to a psychological

stressor" but then makes a number of critical errors in analyzing the disclosure of the Mikawa reference:

1. Mikawa used isolated neutrophils, not whole blood.

2. Mikawa used a fixed quantity of isolated neutrophils; they were not stimulating neutrophils in a whole blood sample as specified in claim 1(a). Such isolation involving centrifugation as detailed under the heading "Isolation of neutrophils" is critically avoided by the method of the invention to preserve neutrophil responsiveness as in vivo. As explained further in the attached Declaration, isolation by centrifugation will remove the vast array of chemicals released in blood during stress exposure and also remove other cells that may influence the production of superoxide. Centrifuging neutrophils would affect the sensitivity. In the context of gaining a measure of effect of psychological stress, significantly the inventors found they could use whole blood samples without reference to leukocyte or neutrophil number.

If anything the Mikawa reference reinforces the teaching of Kang – employ a fixed number of isolated neutrophils for PMA/FMLP-challenge assay – but Kang reported an increase rather than a decrease in superoxide production by neutrophils in response to PMA challenge post-exams in healthy individuals, a result at variance with that to be expected using the method of the invention (compare again Example 4). The use of isolated neutrophils removed automatically any possibility of effect of change of leukocyte number on result (Kang also reported an elevated number of leukocytes and neutrophils associated with stress) and meant that conventional colorimetic assay could be performed, yet the results of such prior art challenge assays are now indicated by the work of the inventors to suffer from lack of physiological relevance.

To return to points already made in the first response in discussing the Kang reference, Examples 1 to 5 of Applicants' specification illustrate consistently lower superoxide production above basal in test whole blood samples following PMA-induced challenge compared with superoxide production in control whole blood samples where the test samples were taken from individuals subjected to a stress factor. Lower PMA-induced superoxide production in test samples is a consistent finding requiring no reference to actual neutrophil numbers.

PMA-induced chemiluminescence in whole blood samples does not correlate with neutrophil numbers (although a range of leukocytes can produce superoxide, neutrophils are responsible for the vast majority of superoxide production in blood). In emphasizing this point, reference is made again to the badger studies as reported in Example 1. It is to be noted that in those studies leukocyte numbers in samples were determined and total superoxide production was measured as superoxide production per 10^9 neutrophils/L. Differences in leukocyte numbers were found between samples from the test animals (transported) and the control animals (not transported) with transported animals having a higher percentage of neutrophils. Nevertheless, whether leukocyte coping capacity was calculated per 10^9 neutrophils/L or equated with chemiluminescence assay of total superoxide production above basal, the same pattern of results was observed: uniform depression of superoxide production in response to PMA challenge (also sometimes referred to as PMA-induced respiratory burst) in test samples from transported animals compared to control samples from non-transported animals (see the bottom paragraph on page 16; the second and third paragraphs on page 18 and Figures 1 and 2 of Applicants' specification). It was these studies that first convinced the inventors that they had a struck upon a highly convenient and physiologically relevant method of quantifying the physiological effect of stress. It is those same studies which are reported in the McLaren reference.

With hindsight, the Examiner is wrongly trying to equate the studies of Mikawa with the studies of Applicants by referring to surgical stress as if the comparison of the ability of a fixed quantity of neutrophils to generate superoxide immediately pre-, during and post-abdominal surgery (assessment of perioperative changes in neutrophils) is relevant to quantifying psychological stress. As already stated above, this is just not scientifically acceptable. Mikawa concluded that abdominal surgery may be associated with neutrophil impairment in neonates and infants. The authors themselves (and many others) report that both anaesthesia and surgery cause neutrophil suppression (note again in Mikawa, the paragraph beginning at the bottom of the second column on the front page). The results presented in Figure 1 of the Mikawa reference allow comparison of superoxide production by a fixed number of neutrophils derived from patients

over the perioperative period surrounding a surgical procedure, commonly taken to include ward admission, anesthesia, actual surgery and recovery. It is not possible to say anything from such results about psychological stress.

It is the case that Example 6 in the original specification relates to studies on individuals entering hospital for surgical procedures. But assessment of psychological stress associated with hospital admissions or with post-operative matters was not a consideration of Mikawa. For a start, this demands the right type of controls.

In summary, Mikawa was just not concerned with the same problem even remotely. Mikawa used isolated neutrophils, fixed quantity of neutrophils, and surgical stress (abdominal surgery) instead of psychological stress.

Pfefferkorn merely provides enhanced luminol chemiluminescence assays for superoxide. While such measurement of superoxide might be used in substitution for colorimetric assay in studies of the type reported in Mikawa, this does not get one any closer to the claimed invention: a rapid, objective and physiologically relevant method of quantifying the effect of psychological stressors relying on neutrophils as biomarkers in whole blood samples. Mikawa discloses that surgery affects leukocyte reactivity. This is interesting for those interested in improving recovery from surgery but had no bearing, and could not have any bearing, on finding an improved way of assessing psychological stress.

Section 103 Rejection in view of Mikawa et al. together with Carlson et al.

The suggested combination of the Examiner is not proper and even if both disclosures are considered, one has merely disjointed teachings which even together do not provide or even suggest the claimed invention: Mikawa providing information on the neutrophil suppression effect of surgery and how to monitor this and Carlson dealing with the entirely different technical field of identifying drugs for relieving depression and /or anxiety. In column 36 of Carlson there is reference to whole animal screening wherein animals are socially isolated or subjected to stress by foot shock. However, there is no suggestion whatsoever to use any form of in vitro assay to look at neutrophil

activation or even more importantly to carry out a neutrophil challenge test with whole blood samples as now claimed.

Previous reliance on Tsukamoto et al.

It would appear from the latest Office Action the Examiner now realizes that the Tsukamoto reference is also not helpful in arriving at the invention. This is now reinforced as correct. Tsukamoto scored neutrophil activation by the NBT technique. They were not looking at residual capacity for superoxide production as might be stimulated by PMA challenge. As dealt with in more detail in Dr Mian's Declaration, Tsukamato was not doing the same as the subject invention but just with a different manner of determining superoxide production. The Examiner has kindly provided an English translation of the Tsukamoto reference. Page 8 of that translation refers to as stimulants bacterial cells of *S. aureus* and endotoxin but it is misleading to think of these as a PMA substitute. They are better thought of as employed to simulate bacterial infection (bacterial cells will stimulate an immune response). They do not activate specific intracellular pathways in the same way as PMA challenge of neutrophils, which the inventors believe mimics the stress effect. Even with use of such stimulants and the Crowd–II conditions, the NBT reduction tests showed no significant difference in neutrophil activation with the control group as confirmed by the legend to Figure 4. Tsukamoto did not measure superoxide production as such and there is nothing that can be gleaned from such studies which is even remotely helpful to arriving at the very different methodology of the invention.

Conclusion

Applicants have struck upon a very convenient manner of quantifying the effect of psychological stress in a physiologically relevant manner (whole blood measurement of what is now commonly termed 'leukocyte coping capacity' equating with capacity for induced superoxide production (e.g., using PMA) This will be residual capacity where leukocytes (principally neutrophils) have previously been activated by stress exposure). It is easy to trawl through the prior art to find key words which are in the specification

but it is the context of such words which is important and whether they are directing in any way towards the combined essential features of the invention. As indicated above, it is contested that arrival at the invention firstly demanded a different mindset compared with those looking at induced superoxide production from fixed numbers of isolated neutrophils or counts of activated neutrophils by the NBT reduction test, even where this was in the context of interest in immunological effects of assumed psychological stressors. The invention is not PMA-induced stimulation of neutrophils but a new concept. Despite more than one trawl through the prior art, the Examiner has failed to find any document which even provides a pointer to that concept. In contrast, there are a number of post-invention, peer-reviewed publications now before the Examiner, and the Declaration of the inventor Dr. Mian, which support that the claimed method is a very different approach to looking at psychological stress from what was known from the prior art. A true invention which is finding acceptance as a new 'gold standard' in the field because it is easy to apply, and convincing in its psychological and physiological relevance.

Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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